

Larval Parasitic Nematodes Infecting Marine Crustaceans in Eastern Canada. 1. Sable Island, Nova Scotia

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ABSTRACT: Sable Island, an emergent sand bar located near 44°N, 66°W, 290 km east of Halifax, Nova Scotia, is a useful site to investigate the life cycle of the sealworm, *Pseudoterranova decipiens*, due to the large number of seals that haul out there, and the presence of sealworm in enclosed ponds on the island. Totals of 5,000 amphipods (*Gammarus oceanicus* and *G. setosus*) and 3,448 mysids (*Neomysis americana*) collected from Wallace Lake, Sable Island, in August 1991 were digested enzymatically to compare levels of infection of sealworm between the 2 invertebrate groups. Three third-stage sealworm larvae and 3 larvae of *Paracuaria adunca* were found in mysids, whereas amphipods were uninfected. Four sealworm larvae also were found in 1,408 intertidal sand hoppers (*Americorchesia megalophthalma*) collected from Sable Island beaches in August 1990 and 1991. One nematode was recovered by digestion of 753 amphipods and the other 3 from dissections of 655 amphipods. Results of the present and other studies indicate that mysids may be more important than amphipods in the transmission of sealworm to fish hosts.

KEY WORDS: sealworm, *Pseudoterranova decipiens*, *Paracuaria adunca*, life cycle, mysid, *Neomysis americana*, amphipod, *Americorchesia megalophthalma*, Sable Island.

Life cycles of parasitic nematodes in the marine environment are generally poorly understood. The sealworm, *Pseudoterranova decipiens* (Krabbe, 1878) (Nematoda: Ascaridoidea), is of economic importance because its large reddish-brown third-stage larva in the flesh of groundfish is esthetically distasteful to consumers. In 1985, the annual cost of sealworm removal and downgrading of infected commercial fish fillets in Atlantic Canada was estimated to be \$25 million (U.S.) (Malouf, 1986).

Definitive hosts of *P. decipiens* are marine mammals, especially seals, and larval stages are reported from over 60 species of groundfish in North Atlantic and adjacent waters (McClelland et al., 1990). The early part of the life cycle, however, is not fully understood. Laboratory evidence suggests that meiofaunal crustaceans, such as harpacticoid copepods, are necessary for early development of the parasite or, at least as a transfer host, while a macroinvertebrate is required for transmission to fish (McClelland, 1990). Macroinvertebrates that have proven susceptible in the laboratory include amphipods, mysids, isopods, cumaceans, mud shrimp, polychaetes, and gastropods (McClelland, 1990). Natural infections were observed in the mysids (*Mysis* and *Erythrops* spp. (Scott and Black, 1960), the polychaete *Lepidonotus squamatus* (Val'ter and Popova, 1974), and the amphipods *Caprella septentrionalis* (Val'ter, 1978), *Marinogammarus obtusatus* (Val'ter, 1987), *Gammarus lawrencianus*, and *Unciola irrorata* (McClelland, 1990).

More recently, Marcogliese (1992a) found *P. decipiens* infecting the mysids, *Neomysis americana*, but lacking in amphipods, *Gammarus oceanicus*, collected from brackish ponds on Sable Island, Nova Scotia. While nematodes were not detected in 2,364 amphipods and 1,462 mysids examined microscopically live or by dissection, 4 larval sealworms were found in 3,950 mysids subjected to pepsin digest in a Baermann apparatus. The nematodes *Paracuaria adunca* (Creplin, 1846) and *Cosmocephalus obvelatus* (Creplin, 1825) (Nematoda: Acuarioidea) were also recovered from mysids by enzymatic digestion. As amphipods were not examined by the seemingly more efficient digestion technique, it was premature to conclude that mysids were more heavily infected than amphipods in Sable Island ponds. In the present study, both amphipods and mysids from Sable Island ponds were screened for parasitic nematodes by enzymatic digestion to permit a more valid comparison of nematode infection levels in the 2 crustaceans. Intertidal sand hoppers, *Americorchesia* (=*Talorchestia*) *megalophthalma*, from the beaches of Sable Island, also were examined by dissection and digestion for nematodes.

Materials and Methods

Sampling area

Sable Island is an emergent sand bar 35 km long and 1.5 km wide located near 40°N, 66°W, 290 km east of Halifax, Nova Scotia, on the region of the continental shelf known as the Scotian Shelf. The island is the site

of the largest breeding colony of gray seals (*Halichoerus grypus*) in eastern North America and is also frequented by harbor seals (*Phoca vitulina*). Both phocids are definitive hosts for *P. decipiens* (Scott and Fisher, 1958). Harbor seals often bask on the edge of Wallace Lake, a brackish pond approximately 2 km long and 0.5 km wide located on the south beach of the island. Larval sealworms were found in three-spined sticklebacks (*Gasterosteus aculeatus*) and four-spined sticklebacks (*Apeltes quadratus*) collected from Wallace Lake and surrounding ponds (Marcogliese, 1992b), indicating that the sealworm life cycle can progress at least as far as the fish host in these ponds. Because they are inhabited by few species of invertebrates (Wright, 1989), these ponds provide a simplistic ecosystem where the life cycle of *P. decipiens* and other marine parasites can be investigated *in situ*.

Sampling and laboratory protocol

Totals of 5,000 amphipods (approximately 80% *G. oceanicus* and 20% *G. setosus*), 3,448 mysids (*N. americana*), and 335 isopods (*Chirodotea coeca*) were collected with dip nets from Wallace Lake (43°55.7'N, 59°58.8'W) in August 1991. A total of 1,408 sand hoppers (*Americorchestia megalophthalma*) was collected with dip nets from exposed beaches of the intertidal area on the north side of the island (43°56'N, 60°01.5'W) at low tide in August 1990 and 1991. Dip nets were used in collection of megalops larvae of the crab *Cancer irroratus* from a shallow water area formed between the beach and a sand bar on the south side of the island during low tide. Sand hoppers ($N = 655$), isopods ($N = 88$), and megalops ($N = 170$) were fixed in cold 5% glycerol in 70% ethanol and subsequently examined for parasites by dissection with a stereomicroscope. All other invertebrates were sorted, counted, and digested in 7 g pepsin, 4 ml concentrated HCl, and 6 g NaCl in 1,000 ml water at room temperature in a modified Baermann apparatus equipped with a 1-mm sieve. The filtrate was examined with a stereomicroscope periodically over 24 hr, and nematodes found were fixed in hot 5% glycerol in 70% ethanol. Nematodes were measured and identified using a Leitz Diaplan compound microscope equipped with a calibrated ocular micrometer.

Results

Nematodes were not detected by dissection of 170 megalops larvae of *C. irroratus*, by digestion of 5,000 *Gammarus* spp., nor by dissection ($N = 88$) or digestion ($N = 247$) of 335 isopods (*C. coeca*). Three sealworm larvae, *Pseudoterranova decipiens*, and 3 *Paracuaria adunca* larvae were recovered by digestion of 3,448 mysids (*N. americana*) from Wallace Lake. Four sealworm larvae were recovered from sand hoppers (*A. megalophthalma*), 1 sealworm by digestion of 753 amphipods in 1991, and 3 from dissection of 655 sand hoppers collected in 1990. Infection levels of sealworm in mysids vs. *Gammarus* spp. from Wallace Lake are not independent (*G*-test with

Williams correction, $P < 0.05$), suggesting that they depend on the type of host.

Seven third-stage larvae of *P. decipiens* found in *N. americana* and *A. megalophthalma* possessed lip primordia, an apical boring tooth, an excretory pore ventral at the base of the lip primordia, a cecal ligament, and a tail mucron. Lengths or positions of characteristic structures are shown in Table 1. Measurements correspond to those of wild-caught and lab-reared *P. decipiens* (McClelland, 1990).

Two third-stage larvae of *P. adunca* from *N. americana* were 2.144 and 3.136 mm in length. Lengths of the buccal cavities were 0.080 and 0.112 mm; the muscular esophagi, 0.279 and 0.415 mm; the glandular esophagi, 0.594 and 0.978 mm; and the tails, 0.071 and 0.087 mm. Nerve rings were 0.096 and 0.135 mm, and excretory pores 0.138 and 0.183 mm from the anterior end. Measurements correspond to those of molting second-stage and third-stage larvae (Anderson and Wong, 1982).

Representative specimens of *P. decipiens* from *N. americanus* (#CMNP1992-0019) and *A. megalophthalma* (#CMNP1992-0020 and -0021), and those of *P. adunca* (CMNP1992-0022) have been deposited in the Canadian Museum of Nature (P.O. Box 3443, Station D, Ottawa, Ontario, Canada K1P 6P4).

Discussion

Larval *Pseudoterranova decipiens* were previously described from the mysid *N. americana* from Wallace Lake, Sable Island (Marcogliese, 1992a). Sealworm abundance ($A = \text{no. of nematodes recovered/no. of hosts examined}$) in the 1991 sample of mysids was 0.0009 ($N = 3,448$), similar to that recorded in a 1990 sample ($A = 0.001$; $N = 3,950$). Natural infections of larval sealworm also were found in the mysids *Mysis* sp. and *Erythrops* sp. from the Bras d'Or Lakes, Nova Scotia (Scott and Black, 1960). These results may tentatively be extrapolated to the open ocean, where mysids may play an important role in transmitting sealworm to fish. In a comparative study of diets of 3 species of flatfish, single third-stage larval sealworms were found in the cephalothorax of 2 *Mysis mixta* from the stomach of American plaice (*Hippoglossoides platessoides*) from Sable Island Bank (Martell, 1992).

The fact that nematodes were not found by digestion of 5,000 *Gammarus* spp. herein, nor by microscopic examination of 2,364 amphipods

Table 1. Characteristic dimensions (in millimeters) of *Pseudoterranova decipiens* from the mysid *Neomysis americana* collected in Wallace Lake in 1991 and from the amphipod *Americorchestia megalophthalma* sampled from Sable Island beaches in 1990 and 1991. Total length (L), lengths of the preventriculus (Pre), ventriculus (Ven), intestinal cecum (I.C.) and tail, and distance of nerve ring (N.R.) from the anterior end.

Host	Length (position) of structure					
	L	Pre	Ven	I.C.	N.R.	Tail
<i>Neomysis americana</i>	8.960	1.054	0.697	0.572	0.231	0.122
	7.392	0.889	0.648	0.445	0.221	0.116
	6.528	0.787	0.483	0.220	0.195	0.071
<i>Americorchestia megalophthalma</i>	4.160	0.648	0.358	—	0.189	0.067
	5.888	0.660	0.420	0.154	0.179	0.118
	4.480	0.681	0.415	—	0.179	0.107
	3.936	0.572	0.333	—	0.156	0.096

the previous year (Marcogliese, 1992a), indicates that amphipods of the genus *Gammarus* may not be hosts of sealworm in Wallace Lake, or that infection levels in these crustaceans were so low that they were undetectable with the sample sizes employed. However, gammaridean amphipods cannot be ruled out as intermediate hosts. Natural sealworm infections were reported in *Marinogammarus obtusatus* (Val'ter, 1987), *Gammarus lawrencianus*, and *Unciola irrorata* (McClelland, 1990). Moreover, 4 sealworms were found in 1,408 sand hoppers collected from beaches on Sable Island. These beaches are frequented by a gray seals, the most important definitive hosts of *P. decipiens* in the Northwest Atlantic (McClelland et al., 1983). The proximity of infected seals to these amphipods undoubtedly promotes contact between infective stages of the parasite with these intertidal amphipods.

The 3 smallest sealworm larvae, all from *A. megalophthalma*, did not possess an intestinal cecum, but the absence of an intestinal cecum in smaller third-stage *P. decipiens* is not unusual (McClelland and Ronald, 1974). While cecal length is not a reliable taxonomic characteristic, the ratio of the length of the ventriculus to that of the combined length of the preventriculus and ventriculus can reliably be used to distinguish *P. decipiens* from *Anisakis* sp. (McClelland and Ronald, 1974). Templeman et al. (1957) state that this ratio is 0.31–0.41 in sealworm. In nematodes from the present study, this ratio ranged from 0.35 to 0.42. Furthermore, even though the cecum appeared to be lacking at times, all nematodes possessed a cecal ligament, which is not found in *Anisakis* sp. Within *P. decipiens*, 3 sibling species have been distinguished based on genetic evidence, but only Type B has been found

in Canadian waters south of Labrador (Paggi et al., 1991).

Marcogliese's (1992a) identification of third-stage *Paracuaria adunca* from the mysid *N. americana* was the first record of this nematode in a marine invertebrate. Mature *P. adunca* are cosmopolitan parasites of piscivorous birds (Wong and Anderson, 1982) and probably occur in herring gulls (*Larus argentatus*) and other fish-eating fowl common on Sable Island. Paratenic hosts on the island include three-spined and four-spined sticklebacks and the nine-spined stickleback, *Pungitius pungitius* (Marcogliese, 1992b).

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